CERTIFICATE OF VERIFICATION

I, Ursula Scherz of Schlesierstr. 8, 81669 München, Germany,

state that the attached document is a true and complete translation to the best of my knowledge of the German patent application 197 19 652.7.

Dated: March 12, 2001

Signature of Translator: Ursula Scherz

URSULA SCHERZ

Translator for the English
language duly registered,
commissioned and sworn in
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Certified translation of a priority document

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Certification of Priority on the Filing of a Patent Application

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First Applicant: Progen Biotechnik GmbH, Heidelberg, Neckar/Germany

Title: Tissue Factor for Supporting Wound Healing

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The attached sheets are a true and exact reproduction of the original documents of this patent application.

München, February 15, 2001

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The President

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Applicant: Progen Biotechnik GmbH
Our File: P 3003 - hu / msl

Tissue Factor for Supporting Wound Healing

The present invention relates to the use of tissue factor for supporting wound healing.

Wound healing is disturbed in many diseases, such as diabetes mellitus, vasculitis, arterial occlusive disease, chronic venous and infected ulcer. There are also major problems in connection with wound healing in the case of innervation impairment such as paraplegia, leprosy, neuropathy, etc., and decubital gangrene of persons in need of care. Also known are weak sutures and wound healing impairment in the case of operations, particularly of the intestines or transplantations of skin or other organs. Up to the present, there are no satisfactory products or means by which it is possible to take steps in the case of the above disturbances.

Therefore, it is the object of the present invention to provide a product serving for countering wound healing.

According to the invention this is achieved by the subject matters defined in the claims.

Thus, the subject matter of the present invention relates to the use of tissue factor for supporting wound healing.

The present invention is based on applicant's insight that tissue factor in wounds of animals leads to the formation of blood vessels and thus to wound healing.

Tissue factor is a transmembrane glycoprotein which binds the blood clotting factors VII and VIIa. An activation of the blood clotting factors X and IX is effected by this bond, so that the blood coagulation is started via the extrinsic path and intrinsic path, respectively. Tissue factor has a molecular weight of 43 to 46 kD. Its primary structure is known as is the gene for tissue factor and its localization on the chromosome (cf. Scarpati, E.M., et al., Biochemistry 26, (1987), 5234-5238).

According to the invention tissue factor is used for supporting wound healing. The expression "tissue factor" relates to a tissue factor of any kind and origin. It may be an animal or human tissue factor. It may also be a fragment of a tissue factor which is capable of supporting wound healing. The tissue factor may have a wild-type sequence. Its sequence can also differ from the wild-type sequence by one or several amino acids. In addition, the tissue factor can be part of a fusion protein.

In a preferred embodiment, the tissue factor is present in the form of an expressible nucleic acid. It may be a DNA and/or RNA, a DNA, particularly a cDNA, being preferred. The above statements made on the tissue factor apply here correspondingly to the nucleic acid.

The expression of the nucleic acid can be achieved as usual. It can be favorable for the nucleic acid, e.g. as a DNA, particularly cDNA, to be present in a vector which is suitable for expression in animal cells. A person skilled in the art is familiar with such expression vectors. For example, they may be virus or plasmid vectors. It is advantageous for the vectors not to integrate into the DNA

of cells but to remain episomally within the cells. By this, a transient expression of the tissue factor is achieved, which is preferred. The nucleic acid can also be controlled as a DNA, particularly cDNA, by a constitutive or inducible promoter. An inducible promoter can be e.g. tissue-, organ- and/or tumor-specific.

It can be favorable for the nucleic acid to be controlled as DNA, particularly cDNA, by the CMV promoter e.g. in the expression vector pcDNA3 (Invitrogen company) or by the SV40 promoter, e.g. in the expression vector pSVK3 (Pharmacia company). Such expression plasmids referred to as pcDNA3-TF (tissue factor) and pSVK3-TF, respectively, also represent a subject matter of the present invention. It can be particularly advantageous for the nucleic acids to be present as DNA, particularly cDNA, in a Sindbis virus replicon vector. Such a vector permits an extremely high expression of the nucleic acid. An example of such a vector is the ELVS vector system from Viagene Inc. An expression plasmid referred to as ELVS-TF (tissue factor) also represents a subject matter of the present invention. For the preparation of an above vector, a person skilled in the art will use known methods. Reference is made to Maniatis, T., et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 1982, by way of supplement.

According to the invention tissue factor is used for supporting wound healing. The expression "wound healing" relates to wound healing of any kind and at any site. It can be normal and impaired wound healing. The latter is found in particular in the case of diseases, such as diabetes mellitus, vasculitis, arterial occlusive disease, chronic venous and infected ulcer. Impaired wound healing is also found in the case of innervation impairment such as paraplegia, leprosy, neuropathy, etc., and decubital

gangrene of persons in need of care. Impaired wound healing will also be given if weak sutures and impaired healing occur after operations, particularly of the intestines and transplantations of skin or other organs.

According to the invention tissue factor is administered in the form of a protein or an expressible nucleic acid to support wound healing. It may be favorable for the tissue factor to be administered in combination with further factors supporting wound healing, such as vascular endothelial growth factor (VEGF). These factors can also be present in the form of proteins and/or expressible nucleic acids. The tissue factor and said factors can be administered simultaneously or successively. The kind of administration of tissue factor alone or together with said factors can orient itself by the site of action, i.e. at the site where wound healing shall take place. For example, it is an obvious thing to treat an area on the body surface locally and one within the interior of the body systemically. Common methods can be used for the administration of tissue factor alone or together with said factors. For the local administration it is e.g. favorable to pack the factor or factors into liposomes or absorb them onto gold particles and apply the liposomes to the corresponding site of the body or shoot the gold particles into the tissue. Furthermore, pharmaceutical compositions are provided for the administration of tissue factor alone or together with said factors, which contain common auxiliary substances, such as carriers, solvents, etc. Such compositions also represent a subject matter of the present invention.

By means of the present invention it is possible to support wound healing. This is of major importance for diseases

accompanied by disturbed wound healing. Examples of such diseases are indicated above. One of them, which is to be mentioned particularly is diabetes mellitus, where it is possible to heal large open wounds located at the extremities by means of the present invention. The present invention makes a major contribution to modern medicine.

Brief description of the drawing

Figure 1 shows the formation of blood vessels in wounds transfected with a tissue factor-expressing vector (a). (b) and (c) are controls.

The present invention is explained by the example.

Example: Preparation of a tissue factor-expressing plasmid and its use for supporting wound healing

(A) The entire translated region (1.8 kb) of the mouse tissue factor gene was integrated into the BamHI site of the multiple cloning site of pcDNA3 (Invitrogen). Thus, this region was controlled by the CMV promoter. The expression plasmid pcDNA3-TF was obtained. In the same way, the coding region (0.7 kb) of the mouse tissue factor gene was integrated in antisense orientation into the EcoRI site of the multiple cloning site of pcDNA3. Thus, this region was also controlled by the CMV promoter. The expression plasmid pcDNA3-TF-AS was obtained. It represents control (b).

(B) 6 mm full thickness wounds each were placed on the backs of three female NOD mice (Bomholtgaard, Denmark) at a distance of 8 to 10 mm. These wounds

were treated with mixtures containing 2 µg pcDNA3-TF, pcDNA3-TF-AS (control (b)) and/or pcDNA3 (control (c)), and 12 µg DOTAP transfection reagent (Boehringer Mannheim) each. The wounds were covered with Ohmann Opraflex.

For proving the formation of blood vessels in the wounds, 300 µl of ink (Nigrosin, Sigma) each were injected into the caudal veins of the mice 6 days and/or 8 days following the administration of the mixtures. Thereafter, the animals were killed and the skin regions with the wounds were examined under a microscope.

It showed that the blood vessels formed in wounds on administration of a tissue factor-expressing vector (a) thus supporting wound healing.

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Claims

1. Use of tissue factor for supporting wound healing.

2. Use according to claim 1, characterized in that disturbed wound healing is concerned.

3. Use according to claim 1 or 2, characterized in that the wound healing in the case of diabetis mellitus, vasculitis, arterial conclusive disease, chronic venous and infected ulcer, innervation impairment, decubital gangrene and weak sutures in the case of operations is concerned.

4. Use according to any one of claims 1 to 3, characterized in that the tissue factor is present as expressible nucleic acid.

5. Use according to claim 4, characterized in that the expression of the nucleic acid is transient.

6. Use according to claim 4 or 5, characterized in that the nucleic acid is a DNA.

7. Use according to any one of claims 4 to 6, characterized in that the nucleic acid is controlled by a constitutive or inducible promoter.

8. Use according to any one of claims 4 to 7, characterized in that the nucleic acid is present in a Sindbis virus replicon vector.

9. Use according to any one of claims 1 to 8, characterized in that the tissue factor is present in a liposome or gold particle.

10. Use according to any one of claims 1 to 9, characterized in that the tissue factor is present in combination with further factors supporting wound healing.

11. Use according to claim 10, characterized in that the factors are present as expressible nucleic acids.

12. Use according to claim 10 or 11, wherein one of the factors is VEGF.

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Abstract of the Disclosure

Tissue Factor for Supporting Wound Healing

The present invention relates to the use of tissue factor supporting for wound healing.

Fig. 1

(a)

(b)

(c)